Affinity tuned XmAb® 2+1 anti-mesothelin x anti-CD3 bispecific antibody induces selective T cell-dependent cellular cytotoxicity of human ovarian cancer cells

AACR 2020
Abstract # 4426
Poster # 5654

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Introduction

- Mesothelin (MSLN) is a tumor-associated antigen highly expressed in ovarian tumors. Current treatment options for ovarian cancer have only modest efficacy and there remains a large unmet need for new targeted therapies. We engineered and affinity-optimized MSLN x CD3 bispecific antibodies in an XmAb® 2+1 Fab2-scFv-Fc format that bind bivalently to MSLN and monovalently to CD3. The affinity of the MSLN-targeting arms was reduced to maximize avid binding to on-target MSLN-expressing cancer cell lines while minimizing reactivity on surrogate cell lines approximating normal tissue.

- We used IHC to score the density of MSLN in ovarian tumors and identify representative on-target cancer cell lines that we used to interrogate selectivity of the reduced affinity 2+1 MSLN x CD3 bispecifics. We found the majority of ovarian tumors expressed high amounts of MSLN while minimal expression was found on normal tissues. OVCAR-8 cells were identified as an appropriate model to represent on-target activity, while SKOV-3, A549 and HT29 were identified as appropriate surrogates of low levels of MSLN found on normal tissues. Reduction of MSLN affinity of MSLN x CD3 XmAb® 2+1 bispecific antibodies led to reduction of T cell activity on off-target cell lines, while retaining on-target activity on OVCAR8. Engineering of selective MSLN x CD3 bispecific antibodies allows for preferential killing of cancer cells while potentially minimizing on-target off-tumor effects that may contribute to dose-limiting toxicities.

MSLN expression on ovarian tumors and correlation with antigen densities on cancer cell lines

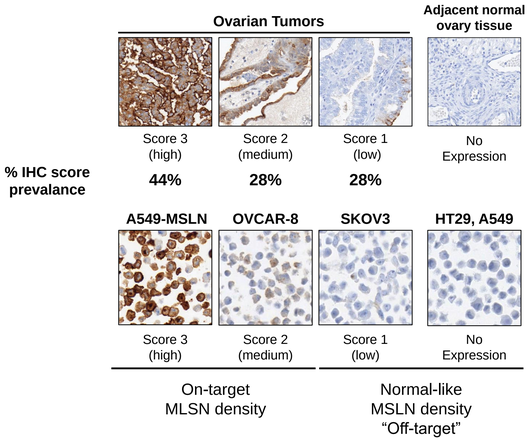

Figure 1: (Top) Illustrative IHC of ovarian cancer biopsy cores and adjacent normal tissue showing mesothelin expression. (Bottom) Illustrative IHC of cancer cell line pellets representing on target and normal-like mesothelin cell surface density.

2+1 MSLN x CD3 enable selective tumor targeting

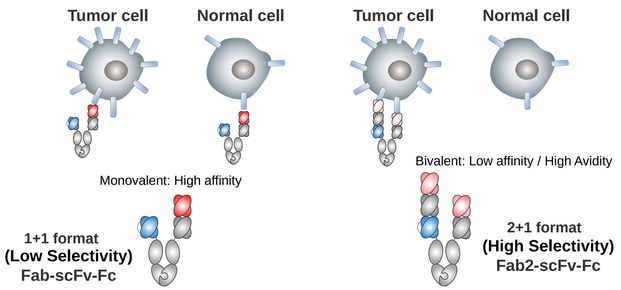

Figure 2: Illustrative 2nd Fab anti-MSLN domain for selective tumor targeting (2+1 format). The 2+1 bispecific format uses a heterodimeric Fc domain, two identical tumor targeting Fab domains and one scFv that targets CD3.

2+1 MSLN x CD3 retain binding despite affinity reduction

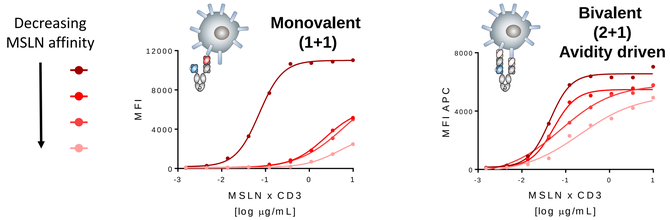

Figure 3: Binding of MSLN x CD3s to high mesothelin expressing OVCAR8 cells. An affinity series was engineered in order to find the optimal selectivity in the 2+1 format.

Quantification of MSLN density on selected surrogate cell lines

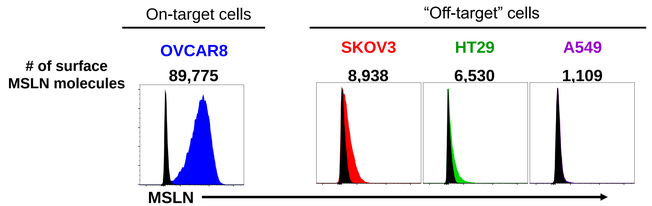

Figure 4: Mesothelin expression on cancer cell lines as MESF determined by flow cytometry.

2+1 MSLN x CD3 exhibit improved on-target selectivity of high mesothelin expressing cells

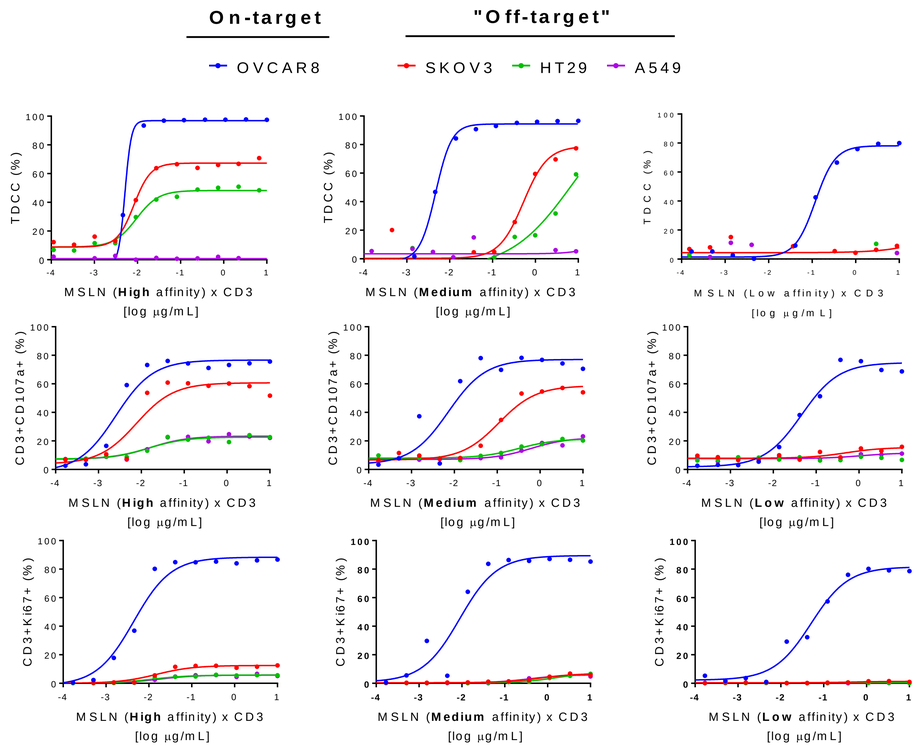

Figure 5: Induction of TDCC (T cell dependent cellular cytotoxicity) (top row), T cell degranulation (middle row) and T cell proliferation (bottom row) by MSLN x CD3s in the presence of cancer cell lines with varying surface MSLN densities. Reduction of MSLN affinity of MSLN x CD3 XmAb® 2+1 bispecific antibodies led to reduction of T cell activity on off-target cell lines, however on-target activity was retained.

Conclusion

The Xmab® 2+1 anti-MSLN x anti-CD3 bispecific shows a promising profile of preferential T cell mediated killing of high MSLN+ cancer lines in vitro but not low MSLN+ cancer lines. This helps potentially avoid systemic toxicity due to on-target off-tumor activation and expansion of peripheral lymphocytes.

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